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Selection and Breeding of King Oyster Mushroom[1]

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Abstract: Three strains of the king oyster mushroom, *Pleurotus eryngii* (DC.:Fr.)Quél., were grown with bottle cultivation system for the evaluation of commercial production. They were the strains ATCC 36047, ATCC 90212 and Holland 150. One of them, i.e. the strain ATCC 36047, showed a high potentiality for commercial production. This strain produced medium to large fruiting bodies with an excellent feature, a firm and fine texture and a long shelf life, while the strain Holland 150 fructified fast and mostly produced large fruiting bodies with a good feature, a soft and fine texture as well as a short shelf life. The latter has a higher biological efficiency than the strain ATCC 36047 did, but it produced many warts on cap surfaces during high RH. In order to obtain a highly productive strain which can produce high quality fruit bodies, monokaryons from the strain ATCC 36047 and Holland 150 were isolated and their mating factors were also determined. Crossing between the monokaryons derived from the two strains and screening of the hybrids thus obtained were conducted. Four highly productive hybrids, i.e. B112, B012, B030 and B011, were obtained. The hybrid B112 produced large fruit bodies with a small cap, an equal to fusiform stipe, a medium soft and fine texture. The hybrid B012 produced stout fruit bodies with a prominent eccentric or lateral stipe and a fan-shaped cap of which surface produced warts during a high humid condition. Its stipe was equal and solid, and consisted of a white and very fine texture. The hybrid B030 produced slender fruit bodies with a medium size, and with a round cap, an equal and solid stipe. This hybrid was inclined to produce many small fruit bodies when the humidity of the cropping room was not properly controlled during cultivation. The hybrid B011 was inclined to produce stout fruit bodies with a large cap and a white and solid stipe. These productive hybrids of the fungus took 54.4 to 60 days from inoculation to the end of crop and they reacted differently to the composition of substrate in the aspect of productivity.

Keywords: Selection, Breeding, King oyster mushroom.

INTRODUCTION

The king oyster mushroom, *Pleurotus eryngii*(DC.:Fr.)Quél., is a typical fungus of the flora of the subtropics and steppes and is widespread in southern Europe and the areas of central Asia and North America

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[10]. It has been valued as the best one of all *Pleurotus* species due to its excellent consistancy of cap and stem, and pleasant aroma as well as culinary qualities[7, 10]. It has also the longest shelf life of any of the other oyster mushrooms due to its thick, firm flesh[8]. Recently the mushroom has been welcomed as a valuable addition to chefs's menus in the United States[6]. Besides, the mushroom has been demonstrated that both fruiting body extract and its powder were effective to repress the increase in blood cholesterol level[1]. The king oyster mushroom would have a great market potentiality in the coming years.

The king oyster mushroom has been commercially cultivated in Taiwan since 1993 using bottle cultivation technology[3, 4, 5]. It has also been successfully grown in Japan in the recent years[1]. Now this new *Pleurotus* species has become a popular mushroom on the traditional markets or supermarkets in the two countries. In order to increase the yield, mushroom quality and biological efficiency of this mushroom strain improvement would play an important role. The mating system of the king oyster mushroom has been shown as homogenic tetrapolar incompatibility system[2], therefore it would be possible to improve the mushroom strain by traditional breeding method. Three strains of the king oyster mushroom were grown with bottle cultivation technology for the evaluation of commercial production and monokayons were isolated from two of them for hybridization. Hybrid dikaryons thus obtained were subjected to a series of fruiting tests. The results are described in this paper.

MATERIALS AND METHODS

Strains of the king oyster mushroom

Strains ATCC 36047, ATCC 90212 and Holland 150 of the king oyster mushroom, ***Pleurotus eryngii*** were used for the evaluation of commercial production. They were maintained on PDA for regular subculturing and stored in liquid nitrogen for a long term storage.

Preparation of original and mother spawn

Sawdust from ***Trema orientalis*** was mixed with 40% of rice bran by weight and the water content of the mixture was adjusted at ca. 63% and its acidity was adjusted at pH 6.5 with calcium carbonate. The mixture was then filled into 200ml flask and sterilized at 121 ℃ for 30 minutes. After cooling down to room temperature the sterilized substrate was inoculated with the mycelium of each king oyster mushroom strain on agar slant and incubated at 25 ℃ for about 25 days. When the mycelium has grown throughout the sawdust substrate in flask, such sawdust culture is called original spawn which can be used to inoculate mother spawn. The mother spawn has the same ingredients of raw material as original spawn, however the substrate was filled in 1100 ml polypropane plastic bottle and sterilized in the same way as cultivation spawn described as following.

Preparation of cultivation spawn for fruiting

Sawdust from ***Trema orientalis*** was used as basic raw material in most experiments. The sawdust was mixed with rice bran or other nutritive supplements in various proportion by volume or by weight. Water content of each mixture was adjusted at 63% to ca. 71% varying with different experiments. These were illustrated in the note below each table of results. The mixtures were filled into 1100 ml polypropane plastic bottles with an automatic filling machine(including filling, pressing, holing and capping), then sterilized at 121 ℃ for one hour. The sterilized substrates were cooled down to room temperature and then inoculated with mother spawn using an automatic inoculator.

Incubation and cropping

The inoculated substrates were incubated at 18.5-21.5℃ for 32 to 42 days depending on the mycelial growth rate of each mushroom strain. After incubation the top layer(ca. 1.5cm in depth) of substrate in each bottle was removed by hand with a special spoon(bottle neck treatment, BNT) or was holed by an automatic holing machine in the center of bottle with a diameter of 1.8cm and a depth of ca. 2cm for fruiting. Then the bottles were moved to a cropping room of which temperature was controlled at 15.5-17.5℃, RH at 90 to 95% and light intensity at ca. 250 lux and carbon dioxide at 800-1200ppm during cropping.

Harvesting and determination of biological efficiency and production efficiency

Mushrooms in each bottle were harvested when the mushroom cap surfaces were flat to slightly up-rolled at the cap margins. In most experiments only one flush of mushroom, was harvested. The harvested mushrooms were then weighed for calculating biological efficiency and production efficiency. Biological efficiency and production efficiency were calculated as following:

Biological efficiency(%)= (the weight of fresh mushrooms harvested ÷ substrate dry matter content before inoculation) X 100

Production efficiency(%) = (the weight of fresh mushrooms harvested ÷ substrate dry matter content before cropping) X 100

Isolation and pairing of monokaryons and cultivation of hybrid dikaryons

One mature fruiting body was harvested from each of the two strains ATCC 36047 and Holland 150 which were cultivated singly in separate cropping houses. Basidiospores were collected separately in a sterilized large petri dish and stored at 2℃ in a refrigerator before use. Isolation of monokayons was conducted by dilution method and confirmed by the absence of clamp connections on mycelium with optical microscope. Monokaryons from each king oyster mushroom strain were paired in all possible combinations on PDA agar slant in test tube for mating type groupping. Seven monokaryons were selected from the strain Holland 150 to cross with those derived from the strain ATCC 36047 on PDA agar slant. The hybrid dikaryons with normal mycelial growth thus obtained were cultivated in bottle using the method described as above. The productive hybrid dikaryons with high qualities of fruiting bodies were subjected to a series of fruiting tests with various substrates of which ingredients were different.

RESULTS AND DISCUSSIONS

Comparisons of productivity and fruiting behavior seen in the strains ATCC 90212, ATCC 36047 and Holland 150 cultivated on a sawdust medium supplemented with 25% of rice bran by volume

The yield of the strain Holland 150 was significantly higher than those of the strains ATCC 36047 and ATCC 90212 including the yields of the first flush and of two flushes no matter what the time of uncapping after complete bottle neck treatment. The strain ATCC 36047 produced heavier first flush mushrooms than the strain ATCC 90212. The strain ATCC 90212 took the least time from bottle neck treatment to the first harvest (Table 1). After bottle neck treatment and recapping, removing the caps after appearance of pinheads or small mushrooms significantly reduced the time for harvesting 85% of total yield and for finishing the harvest of the first flush mushrooms This was resulted from the uniform fruiting caused by recapping treatment. The strain ATCC 36047 produced medium to large fruiting bodies with an excellent feature, a firm and fine texture and a long shelf life(Fig. 1a), while the strain Holland 150 produced mostly large fruiting bodies with a good feature

Table 1. Comparisons of productivity and fruiting behavior seen in the strains ATCC 90212, ATCC 36047 and Holland 150 of *Pleurotus eryngii* cultivated on a sawdust medium supplemented with 25% of rice bran by volume

Strain	Time of uncapping	Yield(g/24bottles)		Days from BNT to 1st picking	Days for harvesting 85% of total yield	Days for finishing the harvest of 1st flush
		1st flush	1st+2nd flushes			
ATCC 90212	BF	2117 e	4143 b	14.3 d	7.3 a	9.2 a
Holland 150	BF	3508 b	4892 a	17.3 b	6.7 a	7.8 ab
ATCC 36047	BF	2742 c	3925 b	16.7 bc	4.8 b	6.3 b
ATCC 90212	AF	2450 d	4141 b	15.8 c	3.0 c	4.2 c
Holland 150	AF	3791 a	4767 a	18.5 a	2.0 c	2.5 c
ATCC 36047	AF	3233 b	4208 b	17.3 b	2.5 c	3.2 c

[z] Sawdust from *Trema orientalis* and rice bran was mixed in a ratio of 3:1 by volume. The mixture was adjusted at pH 6.82 with calcium carbonate, while the water content at 71.91% by weight. The mixture was filled into 1100 ml polypropane bottles with an automatic machine(KPAC-50-2) for sterilization at 121 ℃ for one hour. Each bottles contained about 650g of substrate(wet).

[y] The sterilized substrate was inoculated with an automatic inoculator(RS-2000). All inoculated substrates were incubated at 20-22 ℃ for 32 days.

[x] After incubation the top layer (ca. 1.5cm in depth) of substrate in each bottle was completely removed by hand with a special spoon(bottle neck treatment, BNT). Then all the bottles were moved to a cropping room of which temperature was controlled at 15.5-17.5 ℃, RH at ca. 95% and light intensity at ca.250 lux during cropping.

[w] After bottle neck treatment all bottles were capped again, then four days after the treatment half of the bottles for each king oyster mushroom strain were uncapped for fruiting(BF), lids on the another half of bottles were removed until pinheads or small fruiting body appeared(AF). Each strain had 6 replicates for each uncapping treatment. Each replicates consisted of 24 bottles of substrate.

[v] Means with the same letter of a column are not significantly different at 5% level by Duncan's multiple range test.

and fine but soft texture and a short shelf life (Fig. 1b). The latter strain easily produced many warts on cap surfaces at high RH. The strain ATCC 90212 produced small fruiting bodies with a slender stipe (Fig. 1c), a firm and fine texture and a long shelf life (Table 2). According to these observations the strain ATCC 36047 showed a higher potentiality for commercial cultivation than the other two king oyster mushroom strains.

Determination of the mating types of the monokaryons derived from two *Pleurotus eryngii* strains

There were 23 and 38 monokaryons isolated from the strains ATCC 36047 and Holland 150 respectively. After intra-stock and inter-stock mating the mating types of these monokaryons were shown in Table 3. The result in Table 3 evidently indicates that ***Pleurotus eryngii*** is a tetrapolar heterothallic fungus of which incompatibility factors, A and B, have multiple alleles. This result is consistent with that of Hilbert (1982) and therefore it is possible to improve the agronomic characteristics of the fungus by traditional breeding.

Crossing of monokaryons and cultivation of hybrid dikaryons

Seven monokaryons were selected from the strain Holland 150 to cross all the monokaryons derived from the strain ATCC 36047. Totally 92 hybrid dikaryons with normal mycelial growth were selected for primary fruiting test. Yield, biological efficiency or both biological and production efficiency of each dikaryon, and shape, texture and taste of the fruit bodies produced were observed and recorded(Table 4 and 5). The productive hybrid dikaryons with good qualities of fruiting bodies were selected for the second fruiting tests using equal amount of sawdust from ***Trema orientalis*** and that from ***Liquidamber formosana*** as the basis of

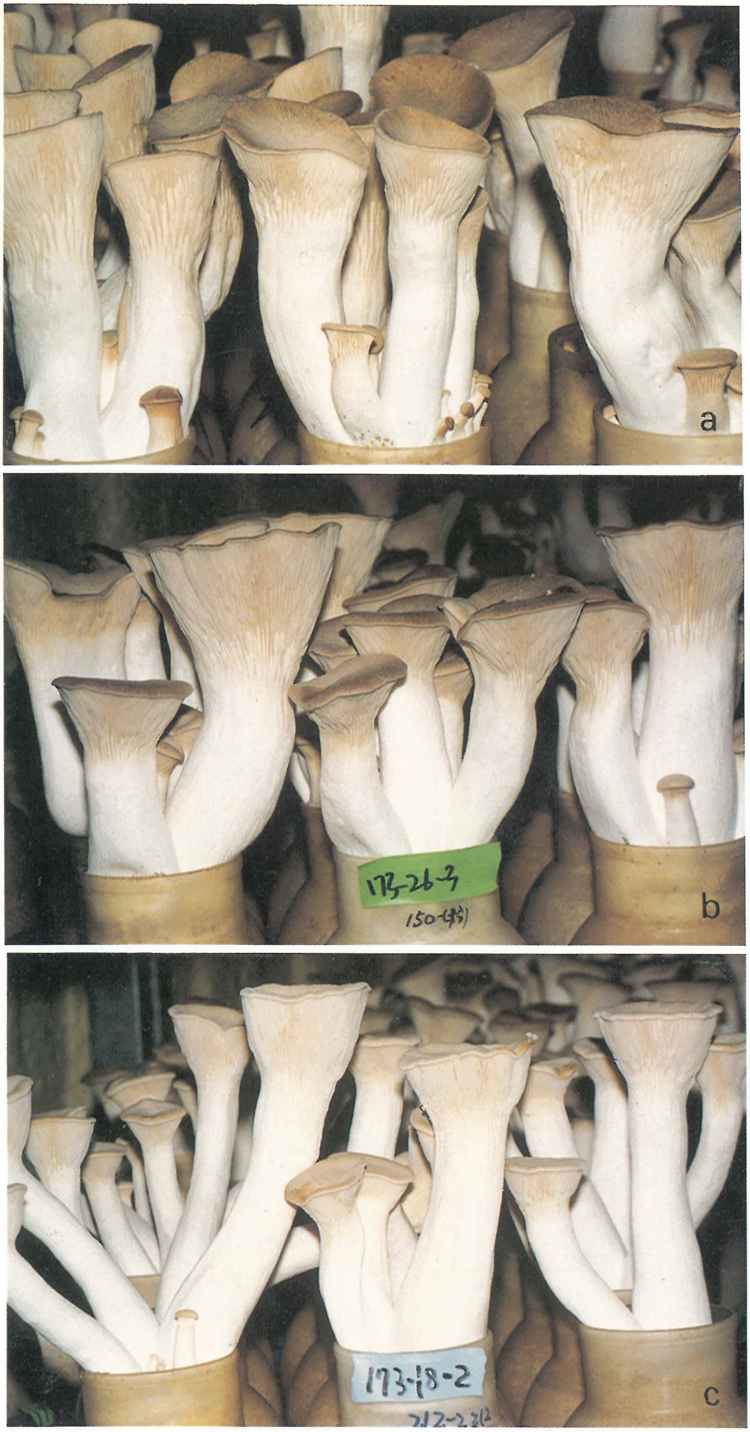

Fig. 1a-c. Fruiting bodies of the *P. eryngii* strains ATCC 36047(a), Holland 150(b) and ATCC 90212(c) grown on a sawdust medium consisted of 35% wheat bran and 15% rice bran by weight.

Table 2. Agronomic characteristics of three *Plearotus eryngii* strains

Items of characteristics	Designation of strains		
	ATCC 36047	Holland 150	ATCC 90212
Earliness of fructification	medium	medium	fast
Size of sporophores	medium>large	medium<large	small>medium
Appearance of sporophores	excellent	good	fair
Warts on cap at high RH	a few	many	few
Color of cap surfaces	light	dark	light
Firmness of tissues	solid	soft	solid
Texture of tissues	fine	fine	fine
Shelf life at 4 ℃	long(>2 weeks)	short(ca.1week)	long(>2 weeks)

Table 3. Mating types of the monokaryons derived from the *Plearotus eryngii* strains ATCC 36047 and Holland 150

Strain	Mating type	Monokaryon
ATCC 36047	A1B1	47003, 47004, 47019, 47023, 47026
	A2B2	47001, 47011, 47012, 47016, 47020
	A1B2	47005, 47009, 47010, 47017, 47021, 47022, 47024
	A2B1	47006, 47013, 47014, 47015, 47018, 47025
Holland 150	A3B3	15002, 15008, 15010, 15025, 15031, 15035
	A4B4	15007, 15013, 15015, 15016, 15023, 15022, 15033, 15036, 15037, 15038, 15040
	A3B4	15001, 15004, 15011, 15012, 15018, 15019, 15026, 15027, 15028, 15029, 15030
	A4B3	15003, 15005, 15006, 15009, 15014, 15017, 15024, 15032, 15034, 15039

Table 4. Primary fruiting test on some hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.B-	Yield (g/bottle)	Biological efficiency (%)	Shape of fruiting body	Texture and taste of fruiting body
15008X47001	8	162.0f-m	67.79 f-m	G[u]	M.soft,sweett
15008X47003	5	172.2c-l	71.95c-l	G	M.soft
15008X47004	1	179.2b-j	74.87b-j	E	M.solid,sweet
15008X47006	7	120.6o-q	50.49o-q	F	M.solid
15008X47008	4	193.6a-c	80.89a-c	F	M.solid,sweet
15008X47010	3	159.0-m	66.43h-m	E	M.solid,sweet
15008X47013	11	180.6b-i	75.56b-i	G	M.solid
15008X47014	12	161.6f-m	67.52f-m	E	Solid, sweet
15008X47019	17	189.6a-f	79.35 a-f	E	M.solid
15008X47021	18	174.4c-l	72.95c-l	E	M.solid,sweet
15008X47022	19	148.0l-n	61.94l-n	G	M.solid
15008X47023	20	151.2j-n	63.28j-n	G	M.solid,sweet
15008X47024	21	193.4a-d	80.19a-d	G	M.solid,TL
15008X47026	22	171.4c-l	72.62c-l	G	M.solid,sweet
15025X47001	23	158.0h-m	67.22h-m	F	M.soft,TL
15025X47008	27	190.0a-f	79.49a-f	F	M.solid,sweet
15025X47009	28	96.8qr	41.55qr	G	M.soft, sweet
15025X47013	30	186.2a-h	78.89a-h	E	Solid, sweet
15025X47016	32	167.4c-m	69.95c-m	E	M.soft,sweet
15025X47019	34	164.4e-m	69.79e-m	G	Solid,sweet

Table 4. Primary fruiting test on some hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150(continued)

Pairing of monokaryon	Dikaryon no.B-	Yield (g/bottle)	Biological efficiency (%)	Shapeof fruit body	Texture and taste of fruit body
15014X47004	36	179.0b-k	74.89b-k	G	M.soft, sweet
15014X47011	40	110.2pq	46.15pq	F	M.solid,sweet
15014X47019	45	173.0c-l	73.38c-l	G	M.solid,sweet
15014X47026	47	149.4lmn	63.29lmn	G	Solid,M.sweet
15023X47001	48	75.8rs	32.77rs	B	Solid, sweet
15023X47003	49	61.2st	26.67st	B	Soft, sweet
15023X47004	51	210.6a	88.99a	G	Soft,M.sweet
15023X47012	58	142.8m-o	59.77m-o	B	M.solid,sweet
15023X47013	59	182.4b-i	77.32b-i	G	M.solid,sweet
15023X47016	62	155.6i-m	65.15i-m	F	M.solid,sweet
15023X47017	63	182.2b-i	77.23b-i	G	Solid.M.sweet
15023X47019	65	187.6a-g	79.48a-g	G	Soft,M.sweet
15023X47026	71	165.0d-m	69.47d-m	B	M.solid,sweet
15029X47001	72	181.4b-i	76.89b-i	G	Soft,M.sweet
15029X47003	73	124.2n-p	52.96n-p	F	M.soft,sweet
15029X47004	74	203.6ab	85.06ab	G	Soft,TL
15029X47005	75	185.4a-h	78.69a-h	F	Soft,TL
15029X47006	76	142.0m-o	61.34m-o	F	M.soft,TL
15029X47007	77	150.6k-n	63.21k-n	F	M.solid,sweet
15029X47008	78	184.4a-h	77.52a-h	F	Solid,sweet
15029X47009	79	141.6m-o	59.16m-o	G	Soft,M.sweet
15029X47010	80	178.0b-k	75.79b-k	G	Soft,sweet
15029X47011	81	171.0c-l	72.54c-l	G	M.soft,M.sweet
15029X47012	82	175.4b-l	73.92b-l	E	M.soft,TL
15029X47013	83	151.0j-n	64.86j-n	F	M.soft,M.sweet
15029X47014	84	155.4i-m	65.36i-m	F	Soft,sweet
15029X47016	86	160.4g-m	67.59g-m	F	Soft,M.sweet
15029X47017	87	189.4a-f	79.98a-f	G	Soft,M.sweet
15029X47019	89	192.2a-e	80.41a-e	G	Soft,M.sweet
15029X47022	91	51.8t	22.51t	B	M.soft,M.sweet
15029X47024	93	211.2a	88.49a	F	M.soft,M.sweet
15029X47025	94	142.6mn	61.79m-o	F	M.soft,M.sweet
15029X47026	95	121.4o-q	50.31o-q	G	Soft,M.sweet

[z]The substrate consisted of 35% of wheat bran, 15% of rice bran and 50% of the sawdust from *Trema orientalis*. Water content of the substrate was adjusted to 65% and pH to 6.5. The substrate was filled into polypropane bottles by an automatic filling machine and sterilized at 120 ℃ for 1.5hrs. Each bottle contained ca. 239.35g of dry matter.

[y]The substrate was inoculated by an automatic inoculator and incubated at 18.5-20.5 ℃ for 42 days.

[x]Before cropping the upmost central part of the substrate in each bottle was holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. After holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90.-96%. The temperature of cropping room was adjusted to 15.5-17.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w]Each dikayon was cultivated in 24-36 bottles, experimental results from the five bottles with higher yield than others were used for statistic analysis.

[v]Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

[u]Shape of fruiting body: G= good; F= fair; E= excellent; B= bad.

[t]Texture and taste of fruiting body: M= medium; TL= Taste Less.

Table5. First fruiting test on the 39 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no. B-	Yield (g/bottle)	Biological efficiency (%)	Production efficiency (%)	Shapeof fruit body	Texture and taste of fruit body
15026X47003	97	184.3e-h	74.60e-h	83.44d-i	G	M.soft,sweet
15026X47004	98	195.3c-e	79.05c-e	87.60c-e	G	M.soft,sweet
15026X47005	99	190.9d-g	77.27d-g	84.90d-g	G	M.soft,M.sweet
15026X47006	100	183.8f-i	74.39f-i	82.37f-j	F	Soft,M.sweet
15026X47007	101	178.8h-j	72.37h-j	80.51g-j	F	Soft,M.sweet
15026X47008	102	180.8g-i	73.18g-i	79.31h-k	G	Soid,sweet
15026X47009	103	169.0j-m	68.40j-m	75.02k-n	G	M.soft,sweet
15026X47010	104	200.5b-d	81.15b-d	89.82a-c	G	Soft,M.sweet
15026X47012	105	185.2e-h	74.96e-h	82.54e-j	F	M.solid,sweet
15026X47013	106	209.3ab	84.72ab	91.33a-c	E	M.solid,sweet
15026X47015	108	167.7k-m	67.88k-m	73.75mn	G	M.soft,sweet
15026X47017	110	193.8d-f	78.44d-f	83.28d-i	E	M.solid,M.sweet
15026X47018	111	200.7b-d	81.24b-d	87.37c-f	G	M.solid,sweet
15026X47019	112	166.0lm	67.19lm	71.62n	E	M.soft,M.sweet
15026X47021	113	190.6d-g	77.15d-g	84.30d-h	G	M.solid,sweet
15026X47022	114	200.2b-d	81.03b-d	87.59c-e	G	M.solid,sweet
15026X47024	116	214.86a	86.97a	92.50ab	G	M.solid,M.sweet
15026X47025	117	173.2i-l	70.10i-l	77.85j-m	E	Solid,M.sweet
15026X47026	118	178.4h-j	72.21h-j	80.64g-j	G	M.soft,sweet
15002X47001	119	205.6a-c	83.22a-c	83.01a	G	M.soft,M.sweet
15002X47003	120	160.7m	65.04m	73.46mn	G	M.solid,sweet
15002X47004	121	186.1e-h	75.33e-h	84.88d-g	G	M.solid,sweet
15002X47005	122	184.1e-i	74.52e-i	83.70d-i	G	M.soft,M.sweet
15002X47006	123	212.0a	85.81a	94.00a	G	Solid,sweet
15002X47007	124	91.3o	36.95o	42.41p	B	M.solid,sweet
15002X47008	127	163.2lm	66.06lm	73.95mn	G	M.solid,M.sweet
15002X47009	125	136.9n	55.41n	62.44o	G	M.solid,M.sweet
15002X47010	126	190.4d-g	77.07d-g	83.86d-i	G	M.solid,sweet
15002X47012	128	187.6e-h	75.93e-h	83.01d-i	E	Solid,M.sweet
15002X47013	129	177.5h-k	71.84h-k	78.94i-l	F	M.solid,sweet
15002X47014	130	162.3m	65.69m	73.96mn	F	M.solid,sweet
15002X47015	131	69.4p	28.09p	32.59q	B	Soft
15002X47016	132	135.4n	54.8n	60.72o	F	M.soft,M.sweet
15002X47017	133	159.0m	64.36m	71.79n	G	Soft,sweet
15002X47018	134	166.7lm	67.47lm	74.50l-n	G	M.soft,sweet
15002X47021	136	194.5d-f	78.73d-f	88.05b-d	E	M.soft,M.sweet
15002X47023	138	191.6d-g	77.55d-g	84.64d-g	G	M.solid,sweet
15002X47025	140	177.8h-k	71.97h-k	79.40h-k	E	M.solid,sweet
15002X47026	141	180.6g-i	73.10g-i	81.01g-j	G	M.soft,sweet

[z]The substrate consisted of 35% of wheat bran, 15% of rice bran and 50% of the sawdust from *Trema orientalis*. Water content of the substrate was adjusted to 65% and pH to 6.5. The substrate was filled into polypropane bottles by an automatic filling machine and sterilized at 120℃ for 1.5hrs. Each bottle contained ca. 247.06g of dry matter.

[y]The substrate was inoculated by an automatic inoculator and incubated at 18.5-20.5℃ for 41 days. Water content of the spawn run substrate was 65.35%.

[x]Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. Ater holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 90-92% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 93.-95%. The temperature of cropping room was adjusted to 15.0-17.0℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 600-800ppm by heat exchanger and CO_2 controller.

[w]Each dikayon was cultivated in 12-24 bottles, experimental results from the ten bottles with higher yield than others were used for statistic analysis.

[v]Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

[u]Shape of fruiting body: G= good; F= fair; E= excellent; B= bad.

[t]Texture and taste of fruiting body: M= medium.

substrate for cultivation. The results were shown in Table 6 and 7. The yields, biological efficiencies and production efficiencies of the most of the hybrid dikayons in the second fruiting test were obviously lower than those of the same dikaryons in the first test. This may be mainly related to the scale of experiment and the basis for statistic data analysis. In the first test only the data of the top 5 or 10 high productive plots were selected for statistic analysis and the repeating cultivation unit was one bottle, while 12 bottles in the second test. In addition, differences in the compositions of substrate might also be one of the reasons, however it is necessary to be confirmed further. Therefore three or five hybrid dikaryons were selected for further fruiting tests using various substrates with different compositions. These dikaryons were selected according to their good appearance and quality of fruit bodies, and higher biological efficiency and production efficiency or earliness of fruiting. The results of cultivation were shown in Table 8, 9, 11 and 12.

Table 6. Secondary fruiting test on 17 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.B-	Yield (g/12 bottle)	Biological efficiency (%)	Production efficiency (%)	Days from holing to 1st.harvest
15008X47024	21	1906.33a	70.40a	76.55a	23.67 bc
15008X47021	18	1779.33ab	68.12ab	72.19ab	22.17 de
15008X47019	17	1759.00abc	64.96abc	70.66abc	25.33 a
15008X47008	4	1753.83abc	64.77abc	69.15bc	19.33 gh
15025X47008	27	1706.33bcd	63.01bcd	67.76bcd	17.00 I
15029X47001	72	1702.00bcd	62.85bcd	68.25bcd	20.50 fg
15023X47004	51	1683.67bcd	62.18bcd	66.91bcde	20.50 fg
15029X47012	82	1682.00bcd	62.12bcd	66.89bcde	19.50 gh
15008X47014	12	1668.33bcd	61.61cd	67.57bcd	22.67 cd
15008X47004	1	1642.27bcde	60.67cd	67.77bcd	24.67 ab
15029X47011	81	1612.33bcde	59.54cde	64.49cde	19.00 h
15023X47017	63	1601.67cde	59.15cde	64.31cde	21.33 ef
15029X47004	74	1564.00def	57.76def	61.35def	17.00 i
15008X47013	11	1495.33efg	54.07efg	60.09ef	19.67 gh
15029X47010	80	1425.00fg	52.63fg	56.59f	22.67 bc
15029X47019	89	1397.00g	51.59g	57.42f	17.67 i
15029X47024	93	1381.00g	51.00g	54.83f	24.00 b

[z]The substrate consisted of 35% of wheat bran, 17% of rice bran, 24% of the sawdust from *Trema orientalis* and same proportion from *Liquidamber formosana*. Water content of the substrate was adjusted to 64% and pH to 6.0. The substrate was filled into polypropane bottles by automatic filling machine and sterilized at 12O ℃ for 1.5hrs. Each bottle contained about 225.6g of dry material.

[y]The substrate was inoculated by automatic inoculator and incubated at 18.5-20.5 ℃ for 41 days. Water content of the spawn run substrate was averagely 65.79%.

[x]Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with a aluminum rod of which diameter was 1.8cm. After holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90-96%. The temperature of cropping room was adjusted to 15-16.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w]Each dikayon had 6 replicates arranged by random, each replicate contained 12 bottles of substrate.

[v]Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

Table 7. Secondary fruiting test on 9 hybrid dikaryons of *Plearotus eryngii* derived from the cross between the strains ATCC 36047 and Holland 150

Pairing of monokaryon	Dikaryon no.	Yield (g/12 bottles)	Biological efficiency (%)	Production efficiency (%)	Days from holing to 1st. harvest
15002X47013	129	2325.33a	85.88a	93.45a	19.50 cd
15026X47019	112	2243.00ab	82.84ab	89.40ab	19.83 c
15002X47001	119	2034.00bc	75.12bc	80.01bc	24.00 b
15026X47013	106	1923.67c	71.04c	83.59abc	27.33 a
15002X47004	121	1877.67c	69.34c	74.48c	24.00 b
15002X47012	128	1470.33d	54.30d	59.25d	28.33 a
15002X47026	141	1452.00d	53.62d	57.02d	17.50 d
15002X47022	114	1413.67d	52.21d	56.69d	27.17 a
15002X47025	140	1114.67e	41.17e	44.03e	23.17 b

[z]The substrate consisted of 35% of wheat bran, 17% of rice bran, 24% of the sawdust from *Trema orientalis* and same proportion from *Liquidamber formosana*. Water content of the substrate was adjusted to 64% and pH to 6.0. The substrate was filled into polypropane bottles by automatic filling machine and sterilized at 12O ℃ for 1.5hrs. Each bottle contained about 225.6g of dry material.

[y]The substrate was inoculated by automatic inoculator and incubated at 18.5-20.5 ℃ for 41 days. Water content of the spawn run substrate was averagely 65.79%.

[x]Before cropping the upmost parts of the substrate in bottles were holed 1.5-2.0cm in depth with after aluminum rod of which diameter was 1.8cm. A holing the bottles were covered with white papers for 4 days. After removing the papers, RH of cropping room was adjusted to 85-90% until small fruit bodies had grown up to 3-5cm high, then the RH was adjusted to 90-96%. The temperature of cropping room was adjusted to 15-16.0 ℃ from beginning to the end of cropping and CO_2 concentration was adjusted to 650-800ppm by heat exchanger and CO_2 controller.

[w]Each dikayon had 6 replicates arranged by random, each replicate contained 12 bottles of substrate.

[v]Duncan's multiple range test, column with the same letter or letters are not significant at 5% level.

Table 8. Comparisons of productivity and fruiting behavior seen in eight *Plearotus eryngii* strains cultivated on a sterilized sawdust medium supplemented with 40% of rice bran

Designation of strain	Yield (g/12 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B112	2615.5 a (218.0)	105.08 a	92.83 a	18.0 b	3.75 d	20.75 c	5.00 c
B030	2430.5 ab (202.5)	103.4 a	86.27 ab	19.0 b	3.50 d	21.50 bc	4.75 c
B012	2425.0 ab (202.0)	98.13 ab	86.07 ab	19.5 ab	3.00 d	21.50 bc	5.25 bc
B011	2258.5 bc (188.2)	91.12 b	80.16 bc	18.0 b	4.00 cd	21.00 c	4.50 c
B045	1998.0 c (166.5)	81.24 c	70.91 c	18.0 b	3.25 d	20.25 c	4.25 c
ATCC 36047	1495.0 d (124.6)	62.47 d	53.07 d	20.5 a	5.75 bc	25.25 a	6.50 abc
Holland 150	1684.0 d (140.3)	69.34 d	60.00 d	16.0 c	8.25 a	23.25 ab	8.75 a
ATCC 90212	1169.5 e (97.5)	48.24 e	41.51 e	18.0 b	6.50 ab	23.50 ab	8.25 ab

[z]The substrate consisted of 40% of rice bran and 60% of sawdust from *Trema orientalis* by weight of dry matter. Water content of the substrate was adjusted to 64% and pH to 6.5. The substrate was filled into bottles by an automatic filling machine and sterilized at 121 ℃ for one hour in an autoclave. Each bottle contained ca. 234.77g of dry matter.

[y]The substrate was inoculated with an automatic inoculator and incubated at 18.5-20.5 ℃ for 34 days.

[x]Before cropping the upmost and central parts of the spawn run substrate in bottles were holed 1.5-2.0cm in depth, 1.8cm in diameter with a PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room (negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration.

[w]Each strain had 4 replicates, each replicate consisted of 12 bottles of substrate.

[v]Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 9. Comparison of the productivity and fruiting behavior seen in seven *Plearotus eryngii* strains cultivated on a sterilized sawdust medium supplemented with 30% of barley flour and 20% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	4348.6 a (181.2)	84.07 ab	82.50 a	15.6 cd	6.2 abc	20.8 ab	6.4 abc
B030	4482.8 a (186.8)	86.47 a	85.05 a	14.0 e	7.2 a	20.2 c	7.4 a
B045	4069.6 b (169.6)	81.95 bc	77.21 b	17.0 ab	4.2 d	20.2 c	4.4 d
B0112	3942.0 b (164.3)	77.82 cd	74.78 b	16.2 bc	5.0 cd	20.2 c	5.4 cd
ATCC 36047	3954.6 b (164.8)	73.90 d	75.02 b	17.8 a	4.2 d	21.0 a	4.2 d
Holland 150	4082.6 b (170.1)	81.71 bc	77.45 b	15.2 cd	5.8 bc	20.0 c	5.8 bc
ATCC 90212	3300.0 c (137.5)	63.03 e	62.61 c	15.0 de	6.4 ab	20.4 bc	6.8 ab

[z]The substrate consisted of 30% of barley flour, 20% of rice bran and 50% of sawdust from *Trema orientalis* by weight of dry matter. Water content of the substrate was adjusted to 64% and pH to 6.5. The substrate was filled into bottles by an automatic filling machine and sterilized at 120 ℃ for one hour in an autoclave. Each bottle contained ca. 219.63g of dry matter.

[y]The substrate was inoculated with automatic inoculator and incubated at 18.5-20.5 ℃ for 34 days.

[x]Before cropping the upmost central part of the spawn run substrate in each bottle was holed 1.5-2.0cm in depth, 1.8cm in diameter with an PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room (negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration.

[w]Each strain had 5 replicates and each replicate consisted of 24 bottles of substrate.

[v]Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 10. Formulas of the substrates for studying the effect of addition of barley flour or wheat bran on the productivities and fruiting behaviors of six *Plearotus eryngii* strains

Substrate no.	Sawdust		Barley flour		Wheat bran		Rice bran		Water added (kg)	Substrate. DW (g/bottle)	Water content before holing(%)
	FW (kg)	DW %	FW (kg)	DW %	FW (kg)	DW %	FW (kg)	DW %			
1	660	50	160.7	35	0	0	69.41	15	239.73	245.33	See note
2	660	50	0	0	162.6	35	68.53	15	224.46	237.22	See note

[z]Sawdust from *Trema orientalis* was heaped for 3 months before use. Water contents of sawdust in the two different substrates were 68.33 and 68.73% respectively. Average water content in barley flour, wheat bran and rice bran were 8.95, 11.15 and 8.95% respectively. The two substrates were added with 3.97kg and 3.92 kg of calcium carbonate respectively. Water content of each substrate was adjusted to 63%. The substrates were filled with an automatic filling machine and sterilized in an autoclave for one hr at 121 ℃. Water contents of spawn run substrate of the strains B012, B045, B112, ATCC 36047, Holland 150 and ATCC 90212 were 63.2, 64.3, 63.53, 64.73,64.53 and 65.20% in the substrate no.1, while 66.27,64.9, 65.06, 66.17, 66.50 and 64.07% in the substrate no.2.

The dikaryons B112, B030 and B012 were not significantly different from each other in yield, production and biological efficiency, however they and the hybrid B011 were significantly more productive than their parents, ATCC 36047 and Holland 150 when the sawdust medium was supplemented with only 40% of rice bran (Table 8). The hybrid B112 produced large fruit bodies with a small cap, an equal to fusiform stipe, a medium soft and fine texture, being suitable for canning (Fig. 2d1-2). It took 56 days from inoculation of substrate to the end of crop. The hybrid B030 produced slender fruit bodies with a medium size, and with a round cap, an equal and solid stipe (Fig.2c1-2). This hybrid was inclined to produce many small fruit bodies in

Fig. 2a-d. Fruiting bodies of the four *P. eryngii* hybrids, B011(a1, a2), B012(b1, b2), B030(c1, c2) and B112(d1,d2) grown on a sawdust medium which consisted of 30% wheat bran and 20% rice bran by weight

one bottle when the humidity of the cropping room was not properly controlled during cultivation. It took 56.75 days from inoculation to the end of crop. The hybrid B012 produced stout fruit bodies with a prominent eccentric or lateral stipe and a fan-shaped cap of which surface produced warts during high humid condition. The stipe was equal and solid, and consisted of a white and very fine texture (Fig.2b1-2). This hybrid took 57.75 days from inoculation to the end of crop. The hybrid B011 was inclined to produce stout fruit bodies with a large cap and a white and solid stipe (Fig. 2a1-2). It took 55.5 days from inoculation to the end of crop.

When the selected hybrid dikaryons were cultivated in a sawdust medium supplemented with 30% of barley flour and 20% of rice bran the hybrids B012 and B030 were found to be significantly more productive than their parents and the hybrid B112 in yield and biological efficiency (Table 9). The hybrid B112 was not significantly different from its parent in their yield, production and biological efficiency. It took 55, 54.4 and 54.6 days for the hybrids B012, B030 and B112 respectively from inoculation to the end of crop (Table 9). The duration for each hybrid was obviously shorter than that for the same hybrid when cultivated on a sawdust medium supplemented with only 40% of rice bran. This might be due to the difference in the composition of substrate and needs to be confirmed further.

In order to examine the effect of different supplements on the productivity of the selected hybrid dikaryons and their parents, two formulas of substrate were used (Table 10). When grown with a sawdust medium supplemented with 35% of barley flour and 15% of rice bran the results of the comparisons of yield, production and biological efficiency were similar to those of the comparison when cultivated with a sawdust medium supplemented with 30% of barley flour and 20% of rice bran except that the hybrid B112 was significantly less productive than its parents ATCC 36047 and Holland 150 (Table 11). When barley flour was substituted by wheat bran in the medium, the yield and biological efficiency of the hybrid B112 was significantly higher than those of the hybrid B012, while not significantly different from those of its parents

Table 11. Comparisons of productivity and fruiting behavior seen in six *Plearotus eryngii* strains cultivated on a sawdust medium supplemented with 35% of barley flour and 15% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	4584.67 a (191.03)	80.21 a	77.87 a	17.83 a	4.33 a	21.17 a	4.83 a
B045	4213.67 b (175.57)	78.95 ab	71.57 b	16.17 b	4.00 a	19.17 b	6.00 a
B112	3495.83 d (145.66)	63.59 d	59.37 d	17.00 ab	4.17 a	20.17 ab	5.17 a
ATCC36047	4066.17 bc (169.42)	74.92 bc	69.06 bc	17.00 ab	4.33 a	20.33 ab	5.17 a
Holland 150	3846.33 c (160.26)	71.80 c	65.33 c	17.00 ab	4.33 a	20.33 ab	5.50 a
ATCC90212	3469.50 d (144.56)	65.28 d	58.92 d	16.00 b	5.67 a	20.67 ab	6.17 a

[z]Formula of the substrates and related data see Table 10.

[y]Substrates were inoculated with an automatic inoculator and incubated at 18.5-20.5 ℃ for 38 days.

[x]Before cropping the upmost central part of the spawn run substrate in each bottle was holed 1.5-2.0cm in depth, 1.8cm in diameter with a PLC automatic holing machine. After holing bottles were covered with white paper for 5days, the cropping room(negative pressure) was controlled at 16-17 ℃ in temperature, 90-95% in RH and under 1050 ppm in CO_2 concentration until the end of cropping..

[w]Each strain had 6 replicates, 24 bottles of substrate in each replicate.

[v]Duncan's multiple range test, columns with the same letter or letters were not significant at 5% level.

Table 12. Comparisons of productivity and fruiting behavior seen in six *Plearotus eryngii* strains cultivated on a sawdust medium supplemented with 35% of wheat bran and 15% of rice bran

Designation of strain	Yield (g/24 bottles)	Production efficiency (%)	Biological efficiency (%)	Days from holing to 1st harvest	Days for harvesting 90% of total yield	Days from holing to harvesting 90% of total yield	Days for finishing harvest
B012	3386.67 c (141.11)	68.21 c	59.48 c	17.33 a	5.17 ab	21.50 a	5.50 b
B045	4094.17 ab (170.59)	79.60 ab	71.97 ab	16.17 abc	4.50 b	19.67 b	5.50 b
B112	3808.67 ab (158.69)	73.47 bc	66.90 ab	15.00 c	5.33 ab	19.33 b	5.67 b
ATCC36047	3708.83 bc (154.53)	72.30 bc	65.15 bc	17.17 a	5.50 ab	21.67 a	6.50 ab
Holland 150	4159.83 a (173.33)	83.13 a	73.06 a	16.67 ab	5.00 ab	20.83 ab	6.17 ab
ATCC90212	2822.67 d (117.61)	52.46 d	49.58 d	15.67 bc	7.17 a	21.83 a	8.00 a

[z] Formulas of the substrates and related data see Table 10.
[y] Other illustrations see Table 11.

(Table 12). The difference in the result of such comparison is obviously ascribed to the difference in the composition of substrate. Since wheat bran contains higher percentage of nitrogen (3.59%) than barley flour (2.01%) (Shiuan-Yun Chien, personal communication) such difference might be related to the content of nitrogen or protein in the substrate for growing the fungus. This result also shows the difference in the preference for nutrient in the substrate as reported by Visscher(9) on other ***Pleurotus*** species. In this study the hybrid B012 seems to prefer a substrate containing less nitrogen or protein and more starch, while the hybrids B112 and one of its parents Holland 150 to prefer a substrate containing more nitrogen or protein and less starch.

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杏鮑菇新品系之選育[1]

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摘 要

三個杏鮑菇品系 (*Pleurotus eryngii*)，即ATCC 36047, ATCC 90212 及Holland 150，以食用菇自動化瓶栽系統栽培評估其商業化生產之價值，試驗結果顯示ATCC 36047品系最具商業栽培潛力，其菇體中至大型，菇形佳，組織堅實，質地細膩，貨架壽命長。Holland 150 品系出菇快，菇體大型，外形亦佳，組織較爲鬆軟且菌傘表面於高溼狀態下易形成細疣，貨架壽命短，但菇體質地細膩，生物效率一般較ATCC 36047爲高。ATCC 90212品系之菇體細長，但菇體組織堅實，質地細膩且貨架壽命長。爲獲得高品質及高產之杏鮑菇新品系，分別自ATCC 36047 及Holland 150品系各分離獲得23及38個單孢菌株，先經單孢菌株自交及兩品系四交配型代表性單孢菌株間雜交，獲知兩品系各單孢菌株之交配型後，經雜交獲得92個菌絲生長正常之雙核體，以一系列不同基質配方栽培比較其產量及出菇特性，獲得四個高產、質優之雜交雙核體，即B112，B012，B030及B011品系。B112品系之菇體大型，傘小，柄多呈紡錘形，菇體組織略軟，但質地細膩。B012品系菇體中大型，菇柄粗短，上下同寬，顯著偏生或側生，傘扇形，其表面相對溼度高時易生疣狀突起，菇體組織白而堅實，質地特別細膩，。B030品系菇體中小型，柄細長上下等寬，空氣溼度高時易生太多子實體，菇體組織白且結實。B011品系易生柄粗傘大之菇體，傘圓，傘緣上翻爲多，菇體組織白而結實。以上品系自接種至採收結束約需54至60天，各品系之產量或生物效率隨栽培基質不同而異。

關鍵詞：杏鮑菇、新品系、選育

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